MECHANISMS, KINETICS AND DESENSITIZATION OF NK-1 TYPE TACHYKININ RECEPTOR ACTIVATION OF PHOSPHOLIPASES C AND A2. J. E. Krause, R. M. Brodbeck, R. Raddatz and B. S. Sachais, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63110

The substance P-stimulated neurokinin-1 receptor (NK-1R) activates inositol phospholipid turnover and arachidonic acid release in both mammalian tissues and transfected cells. In certain cellular situations, the NK-1R also activates cAMP production. Our studies examined response properties of wild type and mutated NK-1R, and explored several mechanisms controlling receptor responsiveness. At the position of a conserved aspartate residue in the second transmembrane domain of the G-protein coupled receptor superfamily, the NK-1R contains a glutamate which was mutagenized to lysine and aspartic acid residues. Radioligand binding demonstrated that the wild type, Asp^{78} and Lys^{78} had substance P binding not significantly different from each other in both K_D and B_{max}, in stably transfected CHO cell lines. The wild type and Asp^{78} receptors had the same ability to activate inositol phospholipid turnover, arachidonic acid release and cAMP production, whereas the Lys^{78} mutant was markedly attenuated in the activation of any of these pathways. Thus, these data demonstrate the importance of residue 78 in NK-1R coupling to cellular effectors. Further studies examined the role of the serine and threonine rich NK-1R carboxyl terminal region by comparing wild type and truncated mutants (MK-1RΔ325). The "tailless" receptor was hyperresponsive in that an 8-10 fold increase in inositol phospholipid turnover per receptor site was observed. Transfection studies of the NK-1R with either NK-2R or human muscarinic m3 receptor were performed to examine receptor responsiveness in repetitive stimulation studies. Substance P-stimulated inositol phospholipid turnover in the presence of LiCl plateaued by 45 min and readdition of peptide did not produce further responses in wild type or truncated receptor expressing cells. However, restimulation of cells with NK-2 or m3 agonists produced further inositol phospholipid responses, suggesting homologous loss of responsiveness. Further stimulation studies were performed after acid wash of cells to remove residually bound substance P. Under these conditions wild type NK-1R desensitized with a $t_{1/2}$=100 min and NK-1RΔ325 desensitized with a $t_{1/2}$=45 min. These studies indicate that the NK-1R carboxyl tail may not serve the same function in receptor regulation as in adenylyl cyclase linked receptors. Overall, both loss of function and gain of function mutants provide insight into substance P-stimulated second messenger responses.

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